GRO-α mRNA Is Selectively Overexpressed in Psoriatic Epidermis and Is Reduced by Cyclosporin A *In Vivo*, But Not in Cultured Keratinocytes

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Interleukin (IL)-8 and gro peptides are members of the intercrine-α family of chemotaxins known to be present in biologically active form in psoriasis lesions. However, the relative contribution of the three different gro genes to the expression of this material is unknown, as is the stimulus for gro overexpression in psoriatic lesions. To address these questions, Northern blot and semiquantitative polymerase chain reaction analysis were performed on RNA extracted from keratome biopsies of normal skin, untreated plaques of psoriasis, or plaques treated for 1 week with low-dose cyclosporin A (CsA). Northern blot analysis revealed a significant correlation between gro and IL-8 mRNA levels in psoriasis lesions from 26 different individuals ($r = 0.61$, $p = 0.0009$), and overexpression of gro was markedly reduced by CsA prior to detectable clinical improvement (79.3%, $p = 0.01$, $n = 22$). To determine which form(s) of gro were overexpressed in psoriatic lesions, total keratome RNA (1 μg) was analyzed by semiquantitative reverse transcription-polymerase chain reaction (SQRT-PCR). In five patients known to markedly overexpress gro and IL-8 mRNAs by Northern blotting, gro-α was approximately six times more abundant than gro-β, and 25 times more abundant than gro-γ. In cultured human keratinocytes, all three forms of gro mRNA were increased by IL-1α or by interferon (IFN)-γ plus tumor necrosis factor (TNF)-α. However, in contrast to the situation *in vivo*, CsA had no inhibitory effect on cytokine-stimulated gro expression in cultured keratinocytes. Taken together, these results demonstrate that the gro-α gene is selectively overexpressed in psoriatic lesions and strongly suggest that overexpression of gro is a keratinocyte response to activated T cells in psoriasis. Key words: gro-α mRNA/psoriasis/cyclosporin A/melanoma growth-stimulatory activity (MGSA). *J Invest Dermatol 101:767–772, 1993*

It has been suggested that several cytokines and growth factors may play important roles in the pathogenesis of psoriasis [1–3]. Interleukin (IL)-8 and melanoma growth-stimulatory activity (MGSA)/gro are heparin-binding polypeptides that display prominent neutrophil and/or lymphocyte chemotactic properties [4–8]. Recently, three different gro genes have been identified, which are tandemly repeated and clustered together with IL-8 and several related cytokines on human chromosomal band 4q21 [9]. As a group, these genes have been called the intercrine-α subfamily [10], which in addition to IL-8 and gro includes platelet factor 4, β-thromboglobulin, and IL-10 [11]. The gro-α, -β, and -γ genes are 85–90% homologous at the protein level, and share with IL-8 and the other intercrine-α family members a cysteine-X-cysteine motif near the amino terminus of the mature polypeptide, leading to their designation as the C-X-C proteins [12]. In contrast, the intercrine-β subfamily of genes, which are clustered on human chromosome 17, are characterized by a cysteine-cysteine motif near the N terminus and have therefore been called the C-C proteins [10–12]. An important property of this family of cytokines is their inducibility by treatment with other, so-called "primary," cytokines, such as IL-1, tumor necrosis factor (TNF)-α, and interferon (IFN)-γ, identifying them as important components of the cytokine cascade [10].

Psoriatic scale was one of the earliest known sources of IL-8, initially called anionic neutrophil activating protein, or ANAP, by Schröder and colleagues [13]. Recently, this group has reported the presence of approximately equal quantities of IL-8–related and MGSA/gro-related proteins in psoriatic scale [14,15]. These gro-related polypeptides display potent neutrophil chemotactic activity [14], suggesting that they may function along with IL-8 to attract infiltrating leukocytes to the epidermis in psoriasis, as well as other inflammatory dermatoses. However, the identity of the gro gene(s) that are expressed in psoriatic lesions is unknown. Therefore, one major aim of these experiments was to determine which form(s) of gro mRNA are overexpressed in psoriatic lesions.

Cyclosporin A (CsA) is an immunosuppressant drug thought to act selectively on the *de novo* activation of T cells [16]. CsA has been confirmed to be a highly effective treatment for psoriasis [17–19], and its efficacy has been advanced as strong evidence for the immunopathogenesis of this disease [20]. One of the primary actions of CsA is to inhibit the production of T-cell–derived cytokines, such as IL-2 and IFN-γ [21]. In turn, these cytokines could participate either directly or indirectly in stimulating the expression of IL-8 and gro in nearby keratinocytes. Thus, IL-8 has been reported to be strongly induced by IL-1 or the combination of IFN-γ and TNF-α in human keratinocytes [22,23], and gro transcripts can be induced by IL-1 and TNF in mammary-derived fibroblasts and epithelial cells [9]. Consistent with this hypothesis, we have recently reported that IL-8 is variably but coordinately overexpressed along

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Abbreviations: DEPC, diethylpyrocarbonate; MGSA, melanoma growth stimulatory activity; SQRT-PCR, semiquantitative reverse transcription-polymerase chain reaction.

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with IL-1β in psoriatic lesions, and that overexpression of both cytokines is rapidly reduced by CsA in psoriatic lesions, but not in cultured keratinocytes [24]. Therefore, to determine whether gro might display similar properties, the second aim of these experiments was to determine whether gro and IL-8 are coordinately overexpressed in psoriasis lesions and reduced by CsA treatment *in vivo,* but not in cultured keratinocytes.

MATERIALS AND METHODS

Patient Selection The 26 patients studied here were a subgroup of 85 chronic psoriatics enrolled in the multidose CsA trial [18]. Twenty-two of these patients received CsA treatment at doses of 3 mg/kg/d (nine patients), 5 mg/kg/d (12 patients), or 7.5 mg/kg/d (one patient), or placebo consisting of olive oil Labrafil base (four patients). For semiquantitative reverse transcription–polymerase chain reaction (SQRT-PCR) analysis of gro-α, -β, and -γ expression in psoriatic lesions, a subgroup of five of these patients was selected on the basis of high IL-8 and IL-1β expression [24]. Two of these patients received CsA at a dose of 5 mg/kg/d and three at a dose of 3 mg/kg/d. Prior to and after 7 d of treatment, lesional buttocks or thigh skin was biopsied using a keratome as described [25]. Informed consent was obtained from each patient, and all studies involving human subjects were approved by the Institutional Review Board of the University of Michigan.

Cell Culture Primary cultures of normal adult human keratinocytes were prepared as described [26]. Cells were treated for 16 h with CsA dissolved in dimethylsulfoxide (DMSO) vehicle (0.1% final DMSO concentration), or with vehicle alone. The cells were then treated for an additional 6 h with IL-1α (100 ng/ml; Dainippon, Osaka, Japan) or a combination of IFN-γ (100 U/ml, Collaborative Research, Bedford, MA) and TNF-α (20 ng/ml, Amgen, Thousand Oaks, CA). After cytokine treatment, cultures were harvested for RNA isolation as described below.

RNA Isolation and Blot Hybridization RNA was prepared from snap-frozen keratome biopsies by the guanidinium isothionate-cesium chloride technique as previously described [25,26]. RNA was isolated from keratinocytes using RNAzol (Tel-Test, Friendswood, TX) as directed by the manufacturer. Forty micrograms total keratome RNA, or 20 μg total keratinocyte RNA (determined by optical density [OD_{260}]) was resolved on 1% formaldehyde-agarose gels and blotted to Zeta-probe nylon membranes (Bio-Rad, Richmond, CA). Blots were hybridized against ^{32}P-labeled probes (see below). Quantitation of blots were performed either by laser densitometry of autoradiograms [25] or by phosphorimager [27]. Gro hybridization signals were normalized to cyclophilin as previously described [25]. Blots were stripped for rehybridization by boiling twice for 20 min in 0.1 × saline sodium citrate/buffer (SSC), 0.5% sodium dodecyl sulfate. Statistical comparisons were made on the normalized data using analysis of variance.

Plasmids and Hybridization Probes Plasmid DNAs were prepared by alkaline lysis and precipitation in polyethylene glycol [25]. cDNA inserts were prepared by digestion with appropriate restriction endonucleases followed by electrophoresis in low–melting-temperature agarose gels. Inserts were ^{32}P-labeled by random priming ($1-4 \times 10^9$ cpm/μg DNA). The IL-8 [24], lipocortin II [25], 36B4 [28], and cyclophilin [25] probes used in these experiments have been previously described. Gro-α, -β, and -γ hybridization probes were prepared from PCR products generated using primers similar to those described by Haskill *et al* [9]. The following primers were used: gro-α forward, 5′-GAA CTG CGC TGC CAG TG-3′, gro-α reverse, 5′-GGC ATG TTG CAG GCT CCT CA-3′; gro-β forward, 5′-CTC AAG AAT GGG CGG AAA GCT-3′, gro-β reverse 5′-CTC TGC TCT AAC ACA GAG GGA-3′; gro-γ forward, 5′-GAA CTG CGC TGC CAG TG-3′ (same as used for gro-α); gro-γ reverse 5′-AGG TGG CTG ACA CAT TAT GG-3′. For PCR, 1 μg of total lesional psoriatic keratome RNA was converted into first-strand cDNA using random hexamers or oligo(dT) primers as described by Kawasaki and Wang [29]. PCR amplification was then carried out on a 5-μl aliquot of cDNA following the same protocol.

Semiquantitative PCR For cDNA synthesis, total keratome RNA (1 μg), β-globin mRNA (1 pg, Gibco-BRL, Gaithersburg, MD), and oligo (dT) (1 μg, Pharmacia, Piscataway, NJ) were mixed in a total volume of 9 μl diethylpyrocarbonate (DEPC)-treated, autoclaved water. This reaction mixture was denatured for 10 min at 70°C, quench-cooled on ice, and 10 μl of 2 × RT-RXN buffer was added. 2 × RT-RXN buffer contained 4 μl 5 × H-RT buffer (Gibco-BRL), 2 μl 5 mM dNTP mix, 2 μl 0.1 M dithiothreitol (DTT), 0.5 μl RNasin (Promega, Madison, WI), and 1.5 μl DEPC-H_2O. After adding 1 μl of Moloney murine leukemia virus (MMLV) reverse transcriptase (Gibco-BRL), the reaction was carried out at 37°C for 1 h, heated to 93°C for 5 min, and held on ice or stored at −20°C until use. Three fivefold serial dilutions of cDNA (i.e., 1:5, 1:25, 1:125) were made in 1 × RT-RXN buffer and amplified by PCR for 23–27 cycles as follows. Each reaction contained 20 μl of diluted cDNA solution, 6 μl of 10 × PCR buffer (Perkin Elmer-Cetus), 100 pmole each of forward and reverse oligonucleotide primers, 0.2 μl (1 unit) Taq polymerase (Ampli Taq, Perkin Elmer-Cetus), and DEPC-H_2O to a total volume of 80 μl. Amplification was for 1.5 min at 94°C (denaturation); 1.5 min at 50–60°C (primer annealing), and 2.0 min at 72°C (primer extension). After amplification, the product of each reaction was subjected to electrophoresis through a 1.5% agarose gel in 1 × TAE buffer as described [30]. The gels were blotted to Zeta-probe membrane (Bio-Rad, Richmond, CA) under alkaline conditions as described by the manufacturer, hybridized against the appropriate combination of ^{32}P-labeled probes under stringent conditions [26], and quantitated by phosphorimager [27]. Size markers (123 ladder; Gibco-BRL) were visualized by addition of 1×10^4 cpm random-primed 123 ladder DNA to the hybridization mix. For comparison of gro, IL-8, and cellular retinoic acid–binding protein (CRABP)-II expression before and after CsA treatment, specific hybridization signals were detected using the cognate PCR product and normalized to β-globin as previously described [31]. For determination of relative abundance of gro-α, -β, and -γ in the same RNA specimens, normalization to β-globin was unnecessary and therefore omitted. In both sets of experiments, a mixture of gro-α, -β, and -γ probes was used for hybridization. In preliminary experiments, each gro PCR product was shown to detect all three products with very similar sensitivity, and very similar results were obtained when the probes were used singly or in combination (data not shown).

The gro primer pair sequences used for semiquantitative PCR were identical to those used for probe isolation as described above. The IL-8, β-globin, and CRABP-II primers were as follows: IL-8 forward, 5′-ATG ACT TCC AAG CTG GCC GTG-3′; IL-8 reverse, 5′-TTA TGA ATT CTC AGC CCT CTT CAA AAA CTT CTC-3′; β-globin forward, 5′-AAG GCT CAT GGC AAG AAG GTG-3′; β-globin reverse, 5′-CTG CAC CTG AGG AGT GAA TTC-3′; CRABP-II forward, 5′-TAC ATC AAA ACC TCC ACC ACC GTG CGC ACC-3′; CRABP-II reverse, 5′-CGT CAT GGT CAG GAT CAG TTC CCC ATC GTT-3′.

RESULTS

Clinical Evaluation As reported previously [18,24], there was no significant difference in the Psoriasis Area and Severity Index (PASI) score after 1 week of CsA therapy in either the entire group of CsA-treated patients or in the cohort of treated patients used for this study. However, with prolonged therapy, marked and significant clinical improvement was observed in the CsA-treated, but not in the placebo-treated, patients [18,24].

Selection of Gro PCR Probes To detect gro transcripts, PCR primers were selected based on sequences unique to each gro gene as previously described [9]. The locations, lengths, and predicted products of these primers are shown in Fig 1*A*. The identity of the products derived was confirmed by digestion with the restriction enzymes Asp700 and Mae1 followed by blot hybridization to a mixed gro probe (Fig 1*B*).

Northern Blot Analysis of Gro Expression in Psoriasis Lesions Because of the high homology between the gro isoforms, and the similarity in the lengths of the cognate transcripts [9], we were unable to determine the pattern of isoform expression from Northern analysis, even using 30-mer oligonucleotide probes designed to be isoform specific (data not shown). Nevertheless, it was still possible to measure the total gro mRNA levels by this technique. Using either gro-α (Fig 2) or gro-γ (not shown) as probes, gro mRNA levels were markedly increased in keratome biopsy specimens from psoriatic lesions; however, the level was variable from patient to patient (Fig 2). In contrast, no hybridization could be observed to the same amount of RNA derived from normal skin using any of the gro probes (data not shown). The same blots were previously hybridized against an IL-8 probe [24], and these results are reproduced for comparison in Fig 2. The patterns of IL-8 and gro hybridization were very similar (Fig 2). The results shown in Fig 2, along with those obtained from an additional ten CsA-treated and four vehicle-treated patients, were quantitated by densitometry and phosphorimager and normalized to the control gene, cyclophilin. This analysis revealed a highly significant positive correlation between pretreatment IL-8 and gro mRNA levels ($r = 0.61$, $p < 0.0009$, $n = 26$, Fig 3).

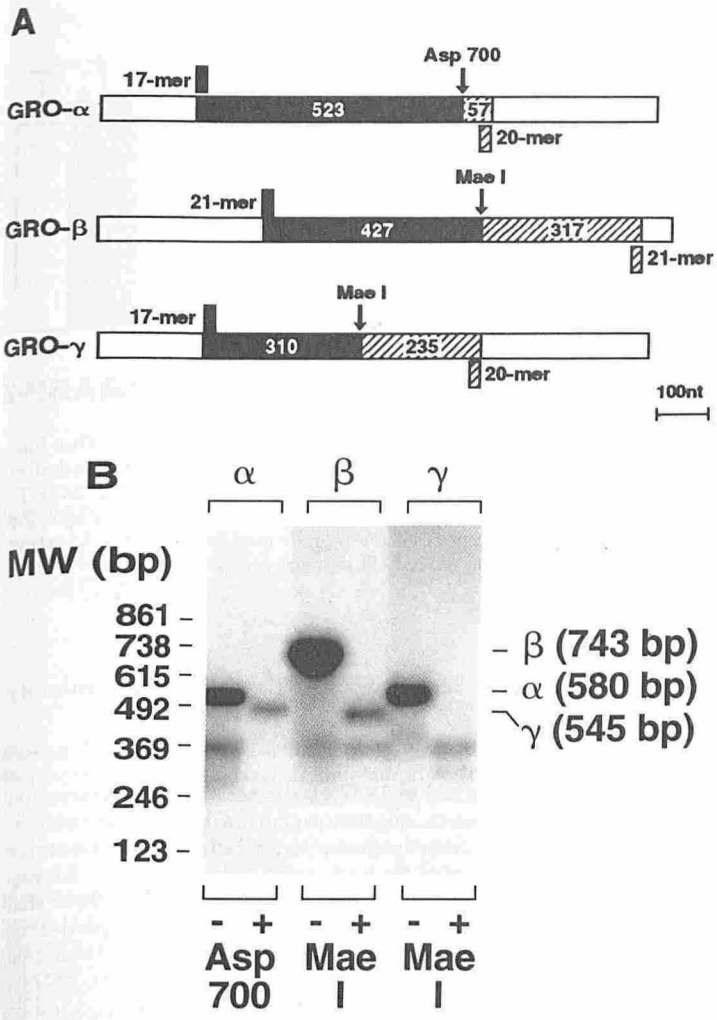

Figure 1. Discrimination of gro isoforms by SQRT-PCR. *(A)* Locations of primers and diagnostic restriction sites. *Bar,* entire length of gro-α, -β, and -γ transcripts. The locations, lengths of forward and reverse PCR primers, and predicted PCR products are shown. Locations of diagnostic cleavage sites for Asp700 and MaeI restriction enzymes are shown by *arrows. (B)* Confirmation of PCR product identity by restriction enzyme digestion. RT-PCR products amplified from lesional psoriatic RNA under non-quantitative conditions were digested with the indicated restriction enzymes, separated on a 1.5% agarose gel, blotted to nylon, and hybridized against a mixture of gro-α, -β, and -γ probes. Loadings of undigested and digested products are not proportional; the *undigested lanes* contain approximately five times as much PCR product as the *digested lanes. Faint lower bands* in the *lanes* containing undigested PCR products reflect nonspecific priming events. Mobilities of 123 ladder size markers are indicated to the *left.*

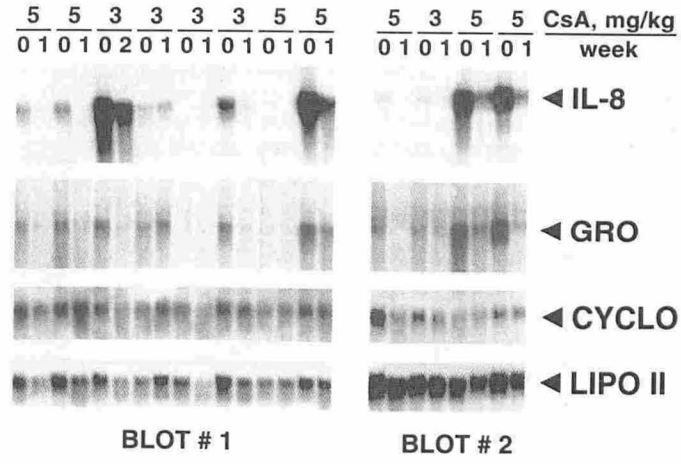

Figure 2. Northern blot analysis of CsA effects on gro and IL-8 mRNA levels in psoriatic skin. Re-hybridizations of two different blots are shown. The results shown for IL-8, cyclophilin, and lipocortin II have been published previously [24] and are included here only for purposes of comparison. Mobilities of ribosomal RNAs are indicated to the *left,* and the hybridization probe used is indicated to the *right* (GRO denotes use of a gro-α probe; identical results were obtained using gro-γ or a mixture of all three gro probes; data not shown). Cyclo, cyclophilin; lipo II, lipocortin II.

A marked reduction in gro mRNA levels was apparent after 1 week of CsA treatment (Fig 2). Quantitation of the blots revealed a 79.3% reduction in mean gro mRNA levels in response to CsA ($p = 0.01$, $n = 22$), which was very similar in magnitude to that previously reported for IL-8 in the same group of patients [24]. In contrast, only a small reduction in CRABP-II expression was seen in the same group of treated patients, as reported previously [24] (Fig 4).

Profile of Gro Isoform Overexpression *In Vivo* Because Northern blotting was unable to distinguish between the gro isoforms, semiquantitative PCR was used to determine the pattern of gro isoform expression in psoriatic lesions. In preliminary experiments, it was determined that the amounts of PCR-amplified gro-α and IL-8 products were proportional to the amount of input RNA when 23 cycles of PCR amplification were used (data not shown). Therefore, 23 cycles of amplification were used in all experiments shown. Under these conditions, the β-globin control showed linear amplification over a range of 0.2–5 attomoles input RNA (data not shown). In RNA extracted from biopsies of normal skin, IL-8 and all three gro isoforms were undetectable under these conditions, whereas 0.2 attomole of β-globin control mRNA was readily detectable (data not shown). In psoriatic lesions, all three forms of gro were expressed. However, all patients consistently expressed gro-α at significantly higher levels than gro-β or gro-γ (Fig 5). By comparison with specific hybridization signals at the highest non-saturating levels of input RNA (7.5 ng loaded, corresponding to 40 ng/reaction), it can be estimated that in untreated psoriatic lesions gro-α mRNA is present at 6.5 ± 1.2 times the level of gro-β (mean $\pm$ SEM, $n = 5$, 95% confidence interval = 3.1–9.9, $p = 0.01$), and 24.7 ± 6.9 times the level of gro-γ (95% confidence interval = 5.4–43.9, $p = 0.03$).

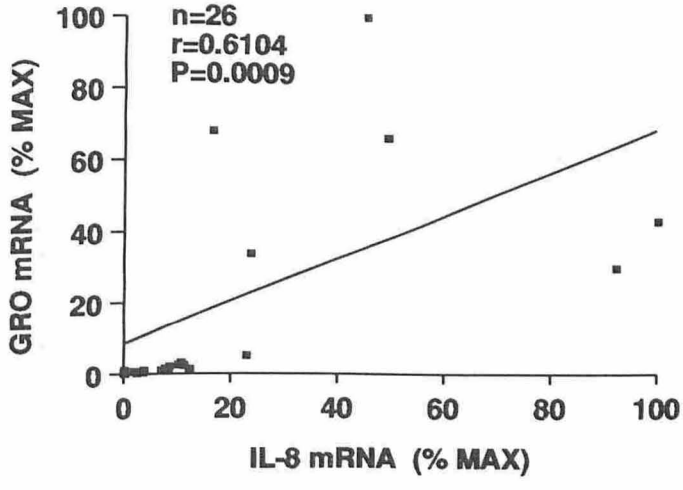

Figure 3. Correlation of gro and IL-8 mRNA levels in lesional psoriatic keratome biopsies. Each *point,* different individual, biopsied at week 0 of the CsA study. The best fit to the data after linear regression analysis is indicated by the *line.*

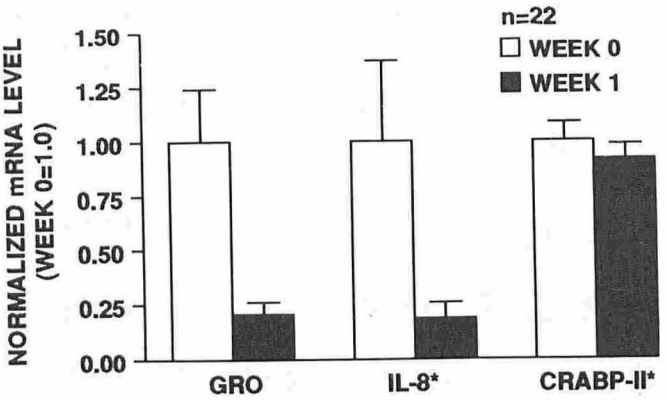

Figure 4. Quantitation of CsA effects on gro mRNA levels in psoriatic skin. *Open bars,* week 0; *closed bars,* week 1. The reduction of gro transcripts at week 1 was significant at the $p < 0.02$ level by a two-sided paired t test. Results of a previously reported [24] analysis of the same group of patients for IL-8 and CRABP-II mRNA levels are indicated by an *asterisk* and are included here for comparison only. *Error bars,* SEM (n = 22).

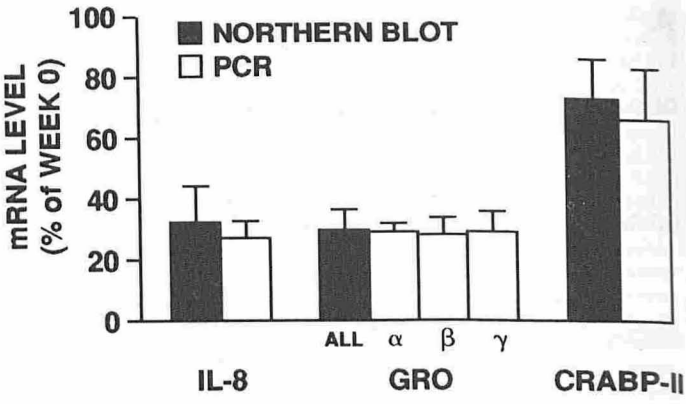

Figure 6. Agreement between PCR and Northern blotting. The same four patients were analyzed for IL-8 and gro mRNA expression before and after CsA treatment. *Solid bars,* quantitative Northern blotting; *open bars,* SQRT-PCR. Transcript levels analyzed by the two methods are given below the figure. *Error bars,* SEM (n = 4). The gro probe used for Northern blotting was a mixture of gro-α, -β, and -γ PCR products as described in *Materials and Methods.*

Effects of CsA Treatment on Gro Isoform Expression Northern blotting and SQRT-PCR were in excellent agreement in comparison of the relative levels of gro, IL-8, and CRABP-II mRNAs before and after 1 week of CsA therapy. All three forms of gro were reduced by the same percentage of their pretreatment values (Fig 6).

Gro Isoform Expression in Cytokine-Stimulated Cultured Keratinocytes Subconfluent keratinocytes in modified MCDB 153 medium (KGM) expressed little if any gro mRNA, as determined by Northern blotting. However, gro transcripts were markedly induced by treatment with 20 ng/ml IL-1α or by 100 U/ml IFN-γ in combination with 20 ng/ml TNF-α (Fig 7*A*). Two bands of about 1.0 *(closed arrow)* and 1.2 kb *(open arrow)* were detectable, and IL-1α treatment consistently resulted in more expression of the 1.2-kb band. As expected, all three PCR-generated gro probes detected similar band patterns. Therefore, the presence of all three gro isoforms in cytokine-stimulated cultured keratinocytes was confirmed by reverse transcription (RT)-PCR (Fig 7*B*). As previously reported for IL-8 [24], overnight pretreatment with CsA (0.1–10 μg/ml) had no detectable inhibitory effect on cytokine-induced gro expression in cultured keratinocytes. In fact, a slight stimulatory effect was noted (Fig 7*A*).

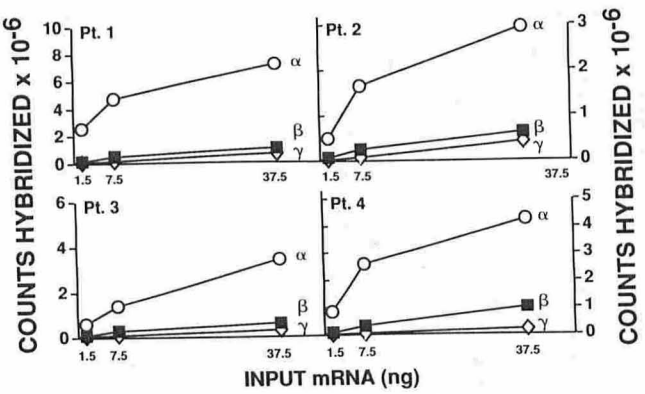

Figure 5. Semiquantitative PCR analysis of relative expression of different gro isoforms in psoriatic lesions. X and Y axis values refer to the amounts of input material analyzed on the gel (15 μl out of 80 μl total reaction volume). Y axis legend refers to net CPM in the band of expected size detected by the phosphorimager. Note different Y axis scales for each patient. Four of five patients analyzed are shown; the results from the fifth patient were similar.

DISCUSSION

Gro was initially identified in 1987 on the basis of overexpression in tumorigenic, relative to nontumorigenic, Chinese hamster fibroblast cell lines [7]. Shortly thereafter, a factor with autocrine growth-stimulatory activity for human melanoma cells was identified and termed MGSA [8]. Shortly thereafter, it became clear that these factors were closely related or identical to a neutrophil-activating protein produced by monocytes [4–6] and IL-1–stimulated dermal fibroblasts [39]. Recently, a search for full-length gro cDNA clones led to the realization that MGSA/gro activity is encoded by three tandemly duplicated genes that are 85–90% homologous at the protein level and are located within the intercrine-α or C-X-C gene cluster of human chromosomal band 4q12–21 [9]. Because of the growth regulatory, chemotactic, and activating activities of gro, it was of interest to investigate gro gene expression in psoriasis, a disease characterized by prominent focal infiltration by neutrophils [32], as well as marked hyperproliferation of keratinocytes [33] and bone marrow-derived cells [34].

The expression of MGSA/gro in the skin was first reported by Richmond and Thomas in 1988 [35]. More recently, Schröder and co-workers have reported the presence of one or more gro-related peptides in psoriatic scale in greatly increased amounts relative to normal heel callus [36]. As shown in Fig 2, we have confirmed the marked overexpression of gro transcripts in psoriatic lesions by Northern blotting. In fact, it was not possible to calculate a -fold overexpression of gro transcripts, as their expression was undetectable in keratome biopsies of normal human skin. Although infiltrating inflammatory/immune cells may be expressing gro transcripts, these cells are too few in number for their transcripts to be readily detectable by the Northern blotting technique. Thus, transcripts encoding the T-cell–derived cytokines IL-2 and IFN-g are undetectable in psoriatic lesions by Northern blotting, although they can be detected by reverse transcription and PCR (unpublished data). Therefore, these results strongly suggest that gro is actually being synthesized by epidermal keratinocytes in psoriatic lesions, and is not deposited in the epidermis by the circulation or infiltrating inflammatory cells. Further *in situ* hybridization experiments will be required to definitively identify the keratinocyte as the primary source of gro expression in psoriatic lesions.

Overexpression of gro in psoriasis lesions was highly variable from patient to patient (Fig 2). In a recent study, we found that

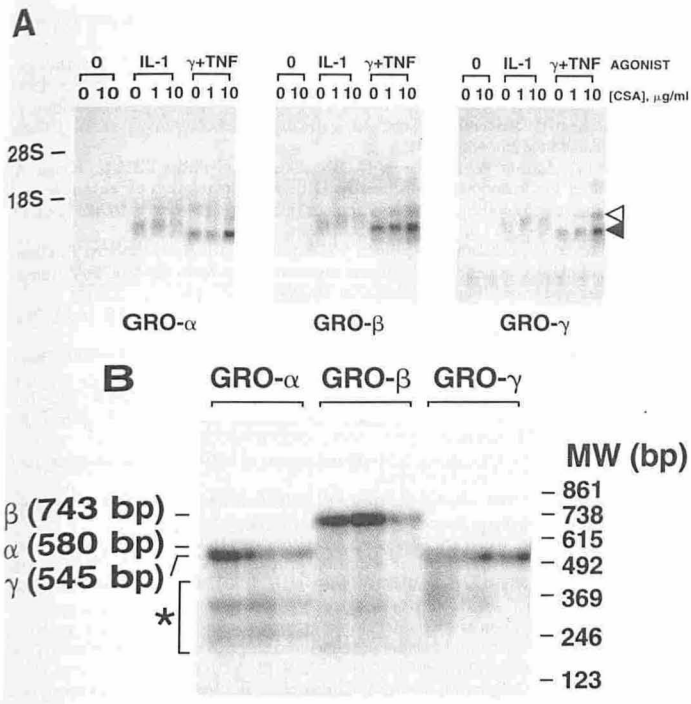

Figure 7. Expression of gro transcripts in cultured adult keratinocytes. *(A)* Northern blot analysis. Normal adult human keratinocytes grown in KGM (~60% confluent) were pretreated overnight (18 h) with or without CsA at the indicated concentrations (0, 1, 10 mg/ml), then stimulated for 4 h with the indicated cytokines (100 U/ml IFN-γ plus 20 ng/ml TNF-α, or 20 ng/ml IL-1α). Total RNA was isolated and 40 μg RNA was loaded per *lane*. Blots were hybridized with the PCR product probes indicated below the panels. *Open* and *closed arrows*, 1.2- and 1.0-kb bands discussed in the text. Approximate mobilities of 18S and 28S ribosomal RNAs are given to the *left*. *(B)* Expression of gro isoforms in cytokine-stimulated cultured keratinocytes. Total RNA was extracted, reverse transcribed, and amplified by PCR. PCR product (15 μl) was blotted and hybridized against a mixture of gro-α, -β, and -γ probes as described in *Materials and Methods*. The *bracket* labeled by the *asterisk* to the *left* of the figure indicates nonspecific products of PCR amplification.

IL-1β and IL-8 were overexpressed in psoriatic lesions in a very similar fashion [24]. On the basis of the uniform and rapid normalization of cytokine expression in response to CsA treatment, we hypothesized that psoriatic keratinocytes are overexpressing IL-8, and possibly IL-1β, in response to stimulation by activated T cells. In this study, the expression of gro and IL-8 transcripts was also highly correlated (Fig 3), and gro overexpression was markedly inhibited by CsA prior to detectable clinical improvement (Fig 4). Taken together, these results strongly suggest that gro expression in keratinocytes is regulated by the same T-cell–dependent stimuli that regulate IL-8. In view of the known ability of IL-1 and IFN-γ plus TNF-α to stimulate IL-8 expression in keratinocytes [22,23], increased expression of these cytokines by T cells or by other cells under their direction could be the trigger for overexpression of gro by psoriatic lesional keratinocytes. Consistent with this hypothesis, both IL-1 α and the combination of IFN-γ plus TNF-α were capable of stimulating gro gene expression in keratinocytes (Fig 7). Alternatively, this stimulus could be provided by direct contact with infiltrating T cells themselves, as suggested by Stoof *et al* [37]. The fact that CsA pretreatment had no inhibitory effect on gro expression in cytokine-stimulated cultured keratinocytes (Fig 7) strongly suggests that CsA does not exert its antipsoriatic effects directly on the keratinocyte. As discussed in the accompanying paper [24], the slight stimulatory effect of CsA on cytokine-stimulated gro expression has also been observed for several other keratinocyte cytokine and growth factor responses in culture, and may in fact reflect a potentiation, rather than an inhibition, of keratinocyte signal transduction in response to cytokine treatment.

Given the recent discovery of three different gro genes [9], these results prompted us to ask which form(s) of gro are overexpressed in psoriatic lesions. Because we were unable to distinguish gro isoform transcripts by Northern blot analysis, we turned to semiquantitative PCR to distinguish between these transcripts, and to determine the effects of CsA on the expression of each isoform. In five patients known to overexpress gro and IL-8 mRNA by Northern blotting, this analysis demonstrated that gro-α was by far the most abundant transcript observed. Consistent with these observations, Schröder and colleagues have independently demonstrated the selective overabundance of gro-α protein in psoriatic scale by amino acid sequencing [36] (J. M. Schröder, personal communication). The fact that all three isoforms were reduced to the same extent after CsA treatment (Fig 6) indicates that all three isoforms may be responsive to the proposed T-cell–dependent stimulus discussed above. The mechanism(s) responsible for the selective overexpression of gro-α in psoriatic lesions remain unknown. All three isoforms appear to be overexpressed to similar extents in cytokine-stimulated cultured keratinocytes (Fig 7*B*); however, more careful analysis is required to document this observation with certainty. By analogy with IL-8 [38], it is possible that gro-α is preferentially expressed by highly differentiated keratinocytes of the upper stratum spinosum.

Taken together, our *in vivo* and *in vitro* data suggest that CsA acts to reduce the production by keratinocytes of multiple proinflammatory cytokines belonging to the intercrine-α family. This mechanism of action appears to involve inhibition of a T-cell–dependent proximal stimulus, but not the ability of keratinocytes to respond to these signals. Moreover, SQRT-PCR appears to be a sensitive and reliable measure of mRNA levels in human skin, of special value when the amount of sample is limited, when the target transcript levels are low, or when highly similar transcripts must be distinguished.

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